Multiple Sclerosis and Related Disorders 43 (2020) 102084

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Original article

Engagement of people with multiple sclerosis to enhance research into the physiological effect of hyperbaric oxygen therapy

Lucy Moore[a,d,1], Paul Eggleton[a,d,*], Gary Smerdon[a,b], Jia Newcombe[a,c], Janet E. Holley[a], Nicholas J. Gutowski[a,d], Miranda Smallwood[a,d]

[a] Institute of Biomedical and Clinical Sciences, College of Medicine & Healthcare, University of Exeter, Exeter, UK
[b] DDRC Healthcare, Hyperbaric Medical Centre, Plymouth, UK
[c] NeuroResource, UCL Queen Square Institute of Neurology, University College London, London, UK
[d] Royal Devon and Exeter Hospital Foundation Trust, Exeter, UK

ARTICLE INFO

Keywords:
Adhesion molecules
Endothelial cells
Oxygen therapy
Public involvement

ABSTRACT

Background: Thousands of people with multiple sclerosis (MS) have used self-administered oxygen therapy in the UK. Clinical trials have been performed, with scant evidence that people with MS have been consulted to explore how they benefit from or how to optimize this treatment. The conventional MS disease disability scores used in trials seldom reflect the effects individuals report when using oxygen therapy to treat their symptoms.
Methods: Three people with MS and the manager of an MS Centre formed a public involvement group and collaborated with clinicians and scientists to inform a lab-based study to investigate the physiological effects of oxygen therapy on microvascular brain endothelial cells.
Results: People with MS often use oxygen therapy at a later stage when their symptoms worsen and only after using other treatments. The frequency of oxygen therapy sessions and hyperbaric pressure is individualized and varies for people with MS. Despite direct comparisons of efficacy proving difficult, most individuals are exposed to 100% O_2 at 1.5 atmosphere absolute (ATA; 1140 mmHg absolute) for 60 min. In a laboratory-based study human brain endothelial cells were exposed in vitro to 152 mmHg O_2 for 60 min with and without pressure, as this equates to 20% O_2 achievable via hyperbarics, which was then replicated at atmospheric pressure. A significant reduction in endothelial cells ICAM-1 (CD54) implicated in inflammatory cell margination across the blood brain barrier was observed under oxygen treatment.
Conclusions: By collaborating with people living with MS, we were able to design laboratory-based experimental protocols that replicate their treatment regimens to advance our understanding of the physiological effects of hyperbaric oxygen treatment on brain cells and their role in neuroinflammation.

1. Introduction

Cells in the body are exquisitely sensitive to oxygen sensing, and oxygen therapy is used to alter cellular physiology in clinical settings (Kirby et al., 2019). The scientists who have contributed to the understanding of the mechanism by which cells adapt rapidly to the changes in the oxygen environment were recently awarded the 2019 Nobel prize for Medicine or Physiology (Burki, 2019). Oxygen therapy has been used as a treatment for MS by over 25,000 people with MS (*pw*MS) who have access to UK registered centres for this treatment over the last 30 years, with over 3 million individual exposures (Eggleton, 2016; James, 2017b). But oxygen therapy is not available through the National Health Service (NHS). The self-use of oxygen as a therapy for MS has been spurred on by some earlier clinical trials. In 1970, a pioneering clinical study was conducted in which 26 individuals with MS were treated with 100% O_2 at 2 atmospheres absolute (ATA) and fifteen (75%) of the subjects showed improved symptoms (Boschetty and Cernoch, 1970). In 1983, the first small double-blinded placebo controlled study produced positive transient improvements in symptoms in 12 /17 (70%) *pw*MS, compared to 1/20 in the control group (Fischer et al., 1983). Participants with varying disease severity were exposed to 100% O_2 at 2 ATA for 90 min. People with less severe disease appeared to respond more effectively.

The following 12 clinical trials failed to allow comparisons of

* Corresponding author.
E-mail address: paul.eggleton@UCB.com (P. Eggleton).
[1] L.M current address: Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK.

https://doi.org/10.1016/j.msard.2020.102084
Received 20 December 2019; Received in revised form 29 March 2020; Accepted 30 March 2020

efficacy between trials as patients were not stratified according to disease severity (which is difficult to do), age/sex or consistency of exposure time to oxygen or pressure regime. Not surprisingly, Bennett and Heard (Bennett and Heard, 2010) concluded that oxygen treatment was ineffective, based on the data available from their meta-analysis of randomized trials using a course of 20 oxygen treatments with non-stratified MS subjects exposed to oxygen at pressures between 1.75 ATA and 2.5 ATA daily for 60–120 min over 4 weeks against a placebo regimen.

There appears to be a lack of knowledge of how oxygen therapy is utilized on an individual level by *pw*MS, including the treatment duration, oxygen pressures, disease status, age of individuals and effectiveness. Furthermore, many laboratory-based studies have not accurately replicated cellular exposure to oxygen based on how *pw*MS use such therapy. To address this knowledge gap, we brought together, researchers and members of the public attending an MS centre to explore the actual experiences of oxygen therapy used by *pw*MS.

Engaging patients and the public in health research can strengthen the quality and efficacy of research studies and successful funding bids increasingly prioritize involving patients and the public in research whereby researchers work 'with' the public not 'on behalf' or 'for' them (Staniszewska et al., 2007). This approach can offer - all stake holders a perspective of symptoms and health service provision, (Brett et al., 2014a, b; Mann et al., 2018; Staniszewska et al., 2012). It also empowers participants engaged in providing or obtaining health services (Gordon et al., 2017; Ross et al., 2005) by promoting research with improved knowledge of outcomes and impact for all concerned (Puts et al., 2017).

We have previously investigated the effect of oxygen exposure under pressure on human endothelial and neural cells at the protein and gene transcription level (Eggleton et al., 2017; Kendall et al., 2012, 2013) and found significant changes in proteins and mRNA involved in inflammation. In this study we investigated the effect of oxygen treatment of adhesion molecule expression on the surface of human brain microvascular endothelial cells using oxygen and pressure regimes recommended and employed by the users of a MS therapeutic centre located in the UK.

2. Materials and methods

2.1. Design

In keeping with the principles of patient and public involvement (PPI), members of an MS centre were invited to collaborate and influence the way the research was planned, conducted and disseminated (Ocloo et al., 2017). The objective was to combine a public involvement approach by establishing a working group to assist with experimental design of laboratory-based experimental research, based on the real-life experiences of pwMS who use oxygen therapy. Then design in vitro oxygen treatment protocols as close to the physiological exposure experienced by individuals with MS, to screen the effect of oxygen treatment on selected physiological parameters of brain endothelia (Fig. 1). In addition, future research in terms of recruitment, ethical issues, development of methods, interview guidance and data collection were considered.

2.1.1. Patient and public involvement

Researchers from the University of Exeter Medical School (PE, LM) and the Research Director of a local Hyperbaric Medical Centre (GS) met with a group of members from an MS Centre where two barochambers provide oxygen therapy. Given that oxygen therapy is not prescribed by clinicians as a treatment for MS (Bennett and Heard, 2010) and is not approved by the National Institute for Health and Care Excellence (NICE) (The Guideline Development Group, 2014) *pw*MS at this centre attend on a private basis and describe themselves as 'members' rather than patients. Some members travel long distances to access treatment.

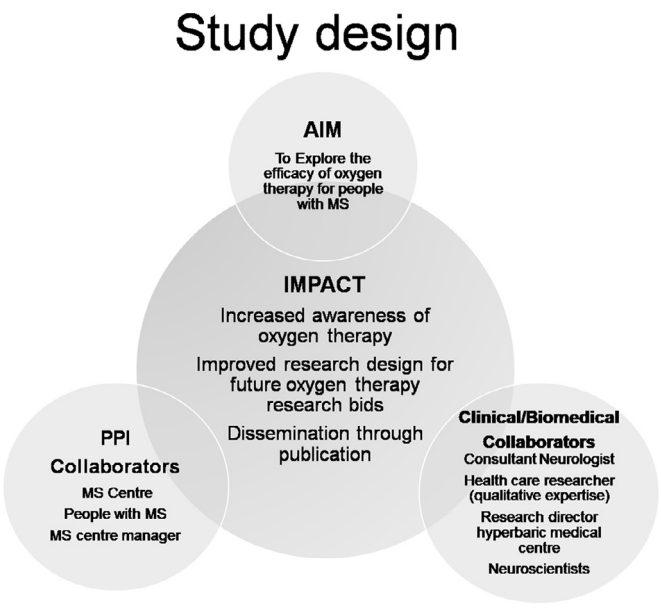

Fig. 1. Study design .

In keeping with the public involvement approach espoused by INVOLVE (Faulkner, 2013) these members were invited to collaborate as consultants and partners to inform the design and development of the study (Brett et al., 2014a, b). This group had no experience of public involvement; therefore the principles and practice of this approach was explained, and supplementary literature and support offered. After consultation, four members of the centre agreed to help with this component of the study and an experienced qualitative researcher (LM) acted as a facilitator. The scientific part of the study was also explained in lay terms with opportunities to ask questions throughout the study. Hyperbaric oxygen therapy delivered under pressure in barochambers was referred to as 'oxygen therapy' by members of the public involvement group and this term will used throughout this paper.

Public involvement meetings were arranged at the centre for ease of access, familiarity and economy. There were four face-to-face 1.5 h meetings, over a 5-month period. In addition, each participant provided additional information by email, as well as reading and amending meeting minutes, allowing flexibility with methods of communication throughout the study. At the end of the study, we visited the MS centre and presented our findings in an open discussion with 50 members.

2.2. Context

2.2.1. Public involvement group collaborative process

This study was conducted over a 5-month period and expectations were discussed with members in the light of this limited time period. Terms of reference and group expectations were agreed at the first meeting with each members' contributions respected and confidentiality and anonymity maintained. Although ethical approval was not required for the public involvement component of this study, the lead for PPI at The NHS Health Research Authority was consulted and an ethical framework followed (Pandya-Wood et al., 2017). Members of the MS centre advised and offered knowledge and expertise, assisting in the planning and design of the study rather than participate in the research.

This public involvement group offered two complimentary perspectives, first they had experiential knowledge of MS and oxygen therapy and secondly, they provided an operational and managerial viewpoint. These members knew each other through management /committee meetings at the centre some as users. They suggested that their interactions with other *pw*MS, who use oxygen therapy at the

centre, afforded opportunities to capture 'other voices' and perspectives. Three members had been diagnosed with MS. Two members had used oxygen therapy for a number of years, and one person had considered starting treatment due to worsening symptoms. The fourth person was the manager of the centre and did not have MS. (2 males and 2 females).

Although members of the group believed in the benefits of oxygen therapy they described the reticence of clinicians to endorse this treatment. Despite this lack of endorsement 10085 individual treatments for *pwMS* were completed from January 2016 to December 2017 at the centre. The public involvement group hoped that their participation would highlight the realities of oxygen therapy for *pw*MS and afford opportunities to share their knowledge and raise awareness. Their preference was that this study would lead to a peer-reviewed publication that combined the public involvement aspects with the scientific component.

2.2.2. Practicalities of oxygen therapy

Oxygen therapy is booked by appointment, weekly in advance and delivered through a face mask for up to 60 min in one of two barochambers, managed by trained staff. All times, dates and 'depths' of treatment for each member are recorded on the centre's secure member database. Some members use oxygen therapy to treat other medical conditions as well as MS. Therapy can be 'personalized' with some people using the therapy at different times of the week and at different depths/pressures depending on how they feel.

Oxygen can be delivered unpressurized if members experience fatigue, claustrophobia, ear problems and other difficulties that prohibit receiving oxygen therapy in a closed and pressurized chamber. Breathing through a masque, at pressure may be difficult for some and the therapy can trigger tiredness and fatigue or stiffness in joints and muscles after sitting for long periods. Despite reassurance from experienced members, up to 50% can drop out of therapy shortly after starting. However, during treatment people keep occupied by reading, listening to music or doing hand exercises as advised by their therapist.

2.3. Human cerebral microvascular endothelial cell (hCMEC) culture

Naturally, pwMS who use oxygen therapy are keen to learn if it has any physiological benefits. One effect oxygen treatment has been reported to have is alteration of adhesion molecules on non-central nervous system (CNS) endothelial cells in ischaemia and reperfusion injury (Namazi, 2008; Song et al., 2016). The inhibition of pro-inflammatory T-cells crossing the endothelial blood brain barrier (BBB) is one mechanism thought to reduce MS pathology. We designed experiments based on the groups' advice to test the effect of oxygen treatment on adhesion molecules levels on a cell line of human cerebral microvascular endothelial cells (hCMEC/D3 cells - VH Bio, UK). This endothelial cell line which is used extensively to model BBB function was cultured and grown to confluence in rat-tail collagen type I coated (100 μg/ml) tissue culture flasks at 37 °C and 5% CO_2 in a humid atmosphere. Cells were cultured in MV-2 endothelial cell growth medium MV supplement Mix (MV-2 - PromoCell, UK). hCMEC/D3 cells were used up to 40 passages. Confluent cells were trypsinized using 0.05% v/v trypsin/EDTA, passaged, sub-cultured and grown in 4% oxygen for 72–96 h. Most *in vitro* cell culture experimentation is performed under atmospheric conditions of 20% O_2, but normal brain oxygen levels have been recorded to range from 4% - 10% (Carreau et al., 2011; Evans et al., 2004). Culture of hCMEC/D3 cells were maintained at 4% O_2. in an oxygen-regulated hypoxia workstation to mimic the conditions of brain cells exposure to oxygen *in vivo* (Sci-Tive UK). Total *in vitro* environment advanced hypoxia workstation, UM025) set at a gas concentration of 4% O_2 and 5% CO_2 at 37 °C. Cells were then exposed to a range of oxygen conditions for 60 mins based on the information provided from the public involvement group discussions. Immunostaining was used to screen for differences in protein expression in cells in culture exposed to different oxygen/pressure conditions and also on tissue sections of brain from MS subjects and control donors. Selected antibodies were used to detect specific adhesion molecules. Namely, (E-selectin); Sigma-Aldrich s9555 clone 1.2B6), Intercellular Adhesion Molecule 1(ICAM); Abcam ab2213 MEM-111: Vascular Cell Adhesion Molecule 1 (VCAM; ThermoScientific MA5-156–36) and Platelet Endothelial Cell Adhesion Molecule (PECAM; Abcam ab76533, a chaperone marker (Calnexin; Thermofisher MA3-027) and tight junction protein (Zho-1; Abcam ab61357).

2.4. In vitro hyperbaric/pressure control treatment

People with MS use oxygen therapy under several pressure conditions ranging from 1.0 to 2.0 ATA. Based on our public involvement group discussions, 100% O_2 used at 1.5 ATA, has been adopted over the past 30–40 years by many *pw*MS as the initial conditions to begin therapy. Breathing 100% O_2 does not result the brain being exposed to 100% O_2, it has been reported that at most, the brain increases oxygen levels from its normal 4% up to 20% after exposure to 100% O_2 under pressure for 60 min (Daugherty et al., 2004; Rockswold et al., 2010). Based on this information, we prepared gas mixtures to allow us to culture brain endothelial cells at oxygen levels that may be reached, during oxygen treatment at MS centres, together with pressure alone and normoxia controls. Specialized mini hyperbaric chambers were made at DDRC Healthcare (Plymouth, UK) to allow us to test cells in culture under similar conditions as *pw*MS. The cell culture test conditions were defined as follows:

(i) Normobaric normoxia (cell culture conditions) - 4% O_2, 5% CO_2, 91% balanced N_2 at 1 ATA, remained in the hypoxic hood and fed with media that had been pre-equilibrated to 4% oxygen stirring for 24 h in 4% oxygen measured using a blood gas analyser (ABL9, Radiometer, UK).
(ii) Normobaric hyperoxia (pressure effect control conditions)– 20% O_2, 5% CO_2, 75% N_2 (= 152mm Hg O_2) at 1 ATA at 37 °C. Aliquots of cells maintained under normbaric normoxia conditions were fed with fresh media and placed in an incubator at 20% oxygen for 1 h at 37 °C.
(iii) Hyperbaric hyperoxia (MS brain exposure conditions) – 13.3% O_2, 3.3% CO_2, 83.4% N_2 at 1.5 ATA absolute (= 152mm Hg oxygen equivalent) at 37 °C. Aliquots of cells maintained under normobaric normoxia conditions were fed with fresh media and placed in a pressurized chamber for 1 h.

All the cells preparations were then returned to normoxia - 4% O_2, 5% CO_2, 91% balanced N_2 at 1 ATA for 24 h, before analysis.

2.5. Immunofluorescence, immunohistochemistry and flow cytometry

Snap-frozen blocks (approximately 1 cm^3) of brain macroscopically normal appearing subventricular deep white matter (WM), active MS lesions and normal control WM samples were supplied from NeuroResource tissue bank, UCL Institute of Neurology, London, UK as previously described (Haile et al., 2017). Tissue was donated for research with informed consent and NHS Ethics Committee approval (Holley et al., 2014). Additional tissue samples and associated clinical and neuropathological data were supplied by the Multiple Sclerosis Society Tissue Bank, funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland, registered charity 207495.

Immunofluorescence was performed using our previously described method (Jung et al., 2018). Briefly, 10 μm thick sections of normal appearing and acute lesions from MS subjects and control WM were probed with primary antibodies diluted 1/100 in PBS, followed by 1/100 dilution of goat anti-mouse Alexa Fluor 647 (far red). Tissue sections were mounted in anti-quenching fluorescent mounting medium with DAPI (ProLong™ Gold anti-fade mountant with DAPI, Fisher

Scientific, P36931). Coverslips were sealed with nail varnish and stored at 4 °C in the dark. Images were visualized using a Leica DM4000 B LED fluorescence microscope. hCMEC/D3 cells grown on chamber slides (Thermo Scientific), were exposed to a range of hyperbaric/pressure control conditions, immunostained with these antibodies and imaged as described above. Examination of MS brain tissue for ICAM and other adhesion molecule expression in specific cell types was performed employing enzyme immunohistochemistry using a Vectastain ABC system® (Vector Laboratories, Peterborough, UK), as described previously (Haile et al., 2017).

For flow cytometry experiments, hCMEC/D3 cells were exposed to experimental conditions (i), (ii) and (iii) (normobaric/hyperbaric/pressure control) conditions, fixed using 1 ml 4% formaldehyde in PBS for 10 min at 4 °C, then the cells were centrifuged at 300x*g* for 5 min. Where appropriate, cells were also permeabilized using 20% v/v methanol at −20 °C for 10 min, centrifuged at 300x*g* for 5 min, re-suspended in 1 ml of blocking solution (10% normal goat serum in PBS) and incubated for 30 min at RT. For flow cytometry, 100 µl of aliquots (2×10^5 cells) were placed in 1.5-ml Eppendorfs tubes and incubated with 40 µl of appropriate primary antibodies added using the manufacturer's recommended concentrations 1:100, for 1 h at 4 °C, followed by incubation with secondary antibody (diluted 1:100) for 1 h at 4 °C. Secondary anti-rabbit (ThermoFisher A11034) or anti-mouse (ThermoFisher A-21,235) antibodies alone or isotype-matched antibodies alone (Mouse:- ThermoFisher 14–4714–82; Rabbit:- ThermoFisher 10500C) were used as controls. All samples were re-suspended in 0.4 ml PBS for flow cytometry analysis employing a Guava easyCyte™ system (Millipore) and the analysis was performed using GuavaSoft 3.1.1 Incyte software.

2.6. Data analysis

During each public involvement group meeting extensive notes were taken by the facilitator and a member of the group to accurately capture all contributions and discussions. These were then sent to all members after the meeting, validated and checked by the group and facilitator. These notes were revisited at each meeting and used to aid further discussions. Statistical analysis on lab-based work was performed using Graphpad Prism 6.0. Results are presented as the mean ± SEM. Two-tailed Student's t tests were performed when appropriate. A significant difference was determined as: $P \leq 0.05$

3. Results

3.1. The public involvement group drew on independent information to make an informed decision about oxygen therapy

Throughout the public involvement collaboration there were opportunities to share different literature, blogs and scientific papers to aid understanding of various perspectives across disciplines within the group and with researchers. This gave an insight into the literature sourced by members that supported their choice of oxygen therapy and explained the history of oxygen use (James, 2017a). Sharing observations and experiences of oxygen therapy helped uncover the practicalities of treatment, the multifactorial nature of why *pw*MS choose oxygen therapy and the perceived benefits.

In addition to physical improvements in movement, measured quantifiably, for example, picking up a cup, the group reported back that many of the 70 members of the centre who routinely used oxygen therapy cited improvements in bladder control, mood, sleep and a reduction in muscle spasms post therapy **(**Fig. 2**),** but only after completing the three week intense course as outlined in Fig. 3, followed by regular 'top-up' sessions on a weekly basis. Although the physical improvements reported by *pw*MS are anecdotal, similar improvements have been reported in many individuals using the 60 or more MS treatment centres in the UK. Despite the reported benefits seldom being documented or shared between these centres, Kochhar and Sangwan (Kochhhar and Sangwan, 2014), reviewed and summarized data collected from several of these centres in previous clinical hyperbaric oxygen trials undertaken by UK neurologists of ~100 *pw*MS. These revealed a reduction in fatigue (70/100), better speech (64/99); better balance (59/100); improved bladder control (68/98) and better locomotion (77/100). With the non-responders either recording no change or feeling worse. In previous hyperbaric oxygen trials, the classification of non-responders was rather vaguely defined, as there was no absolute stratification of subjects based on disease severity. For example, the intensity of glial scarring in each subject tested. Nor was it clear if 'non-responders were unresponsive in all or one disease category being monitored. Nevertheless, hyperbaric oxygen appears to relieve general symptoms of pathology in the bladder and CNS independent of MS. Treatment of seven of eleven non-MS patients, undergoing a 10–20 day course of hyperbaric oxygen therapy for persistent bladder cystitis complications, observed reduced bladder pain, urgency and frequency for up to 12 months (Tanaka et al., 2011). In a randomized control trial of radiation induced cystitis patients. Hyperbaric oxygen reversed macroscopic changes in the bladder induced by the trauma of radiation (Oscarsson et al., 2019). Furthermore, recent oxygen treatment clinical trials of soldiers with mild brain trauma (Walker et al., 2018) and children with cerebral palsy (Long et al., 2017) were shown to improve sleep. Oxygen treatment can also help people to feel empowered by taking back control. Members believed this perspective needed to be captured and fully understood. Moreover, the perspective of spouses and relatives are important as they are the first to observe improvement of symptoms and mood. For this reason, further studies should consider including the perspective of spouses and relatives during interviews. Oxygen therapy can also be a sociable experience where members receive treatment together and talk to each other within the chambers when going to and from pressure.

There are commonalities and differences amongst members who use oxygen therapy at the centre. The public involvement group recognized they were positive in their outlook, whereas others can experience low mood, depression and anxiety as a result of MS. Moreover, the decision to choose this method of treatment and the perceived benefits are as individual as each person's experience of living with MS. Oxygen therapy may be less effective over a number of years, but people still use the treatment because they believe it can slow the progression of MS. However, the majority of *pw*MS tend to seek out oxygen therapy at a later stage, of their disease progression when symptoms worsen, rather than start early when the treatment may be more effective.

3.2. Development of a mind map to describe effects of oxygen therapy

During discussions the public involvement group developed a Mind Map that provided valuable insights into the experiences of using oxygen therapy for members at the centre. Such mind maps are useful for analysing discussions from personal perspectives and in capturing research themes (Whiting and Sines, 2012). This map was individually completed and then combined with maps developed from other members of the group (Fig. 2). This final version took into consideration members' experiences as well as managerial and technical aspects.

This map helped guide the research design including any future research in terms of recruitment, feasibility and logistics of data collection and the development of qualitative methods that are relevant and embedded in real world experiences (Green, 2016). The meetings also provided information concerning the number of people using oxygen therapy, how records are recorded, and the way treatment is used at the centre (see Fig. 3).

3.3. Effect of oxygen treatment on changes in selected adhesion molecules associated with brain microvascular endothelial cells

Part of the public involvement group discussions between

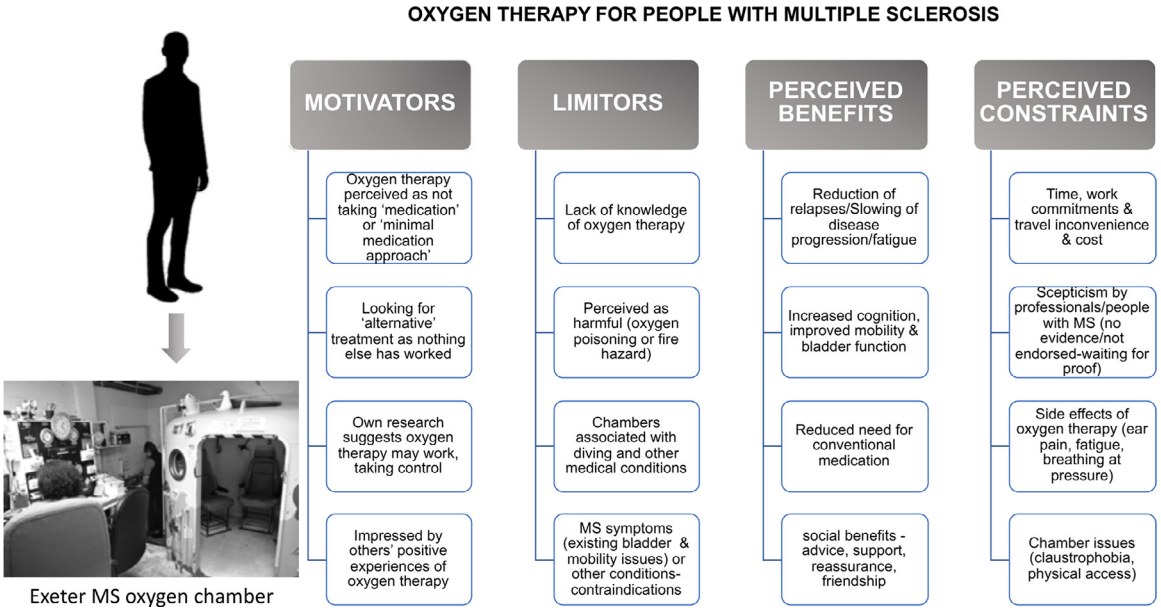

Fig. 2. Public involvement group mind map of perceptions of oxygen therapy.

individuals with MS and our biomedical team raised several important questions; - how does oxygen affect the physiology of the brain? How much additional oxygen enters the brain during treatment? Based on these discussions, a protocol was designed to simulate the amount of oxygen cells are exposed to pre- and during treatment (Fig. 4). Human cerebral microvascular endothelial cells were chosen as the test cell, as they are a major component of the BBB that prevents inflammatory cell migration across the BBB into the brain. To some extent, BBB integrity

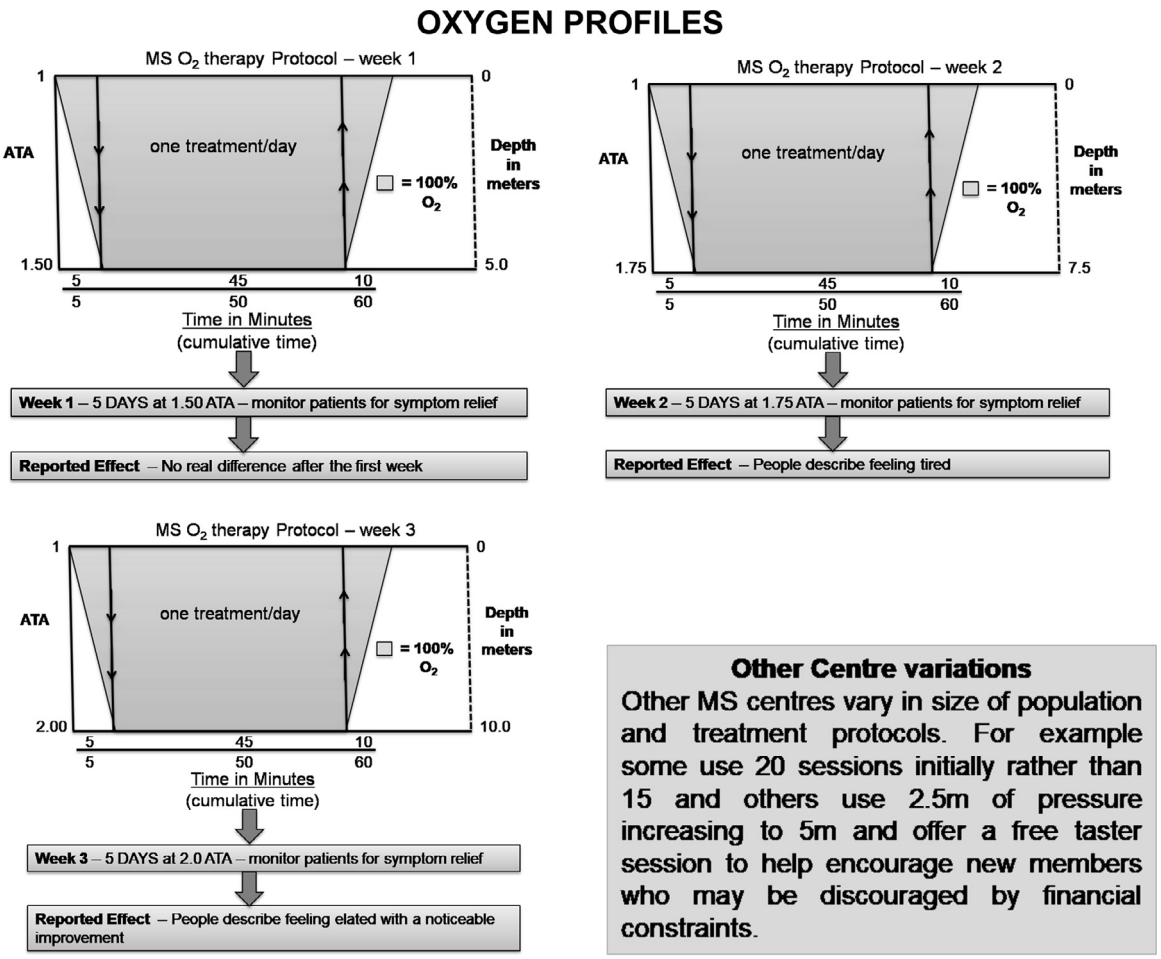

Fig. 3. A typical oxygen treatment regime used to treat MS at an MS centre.

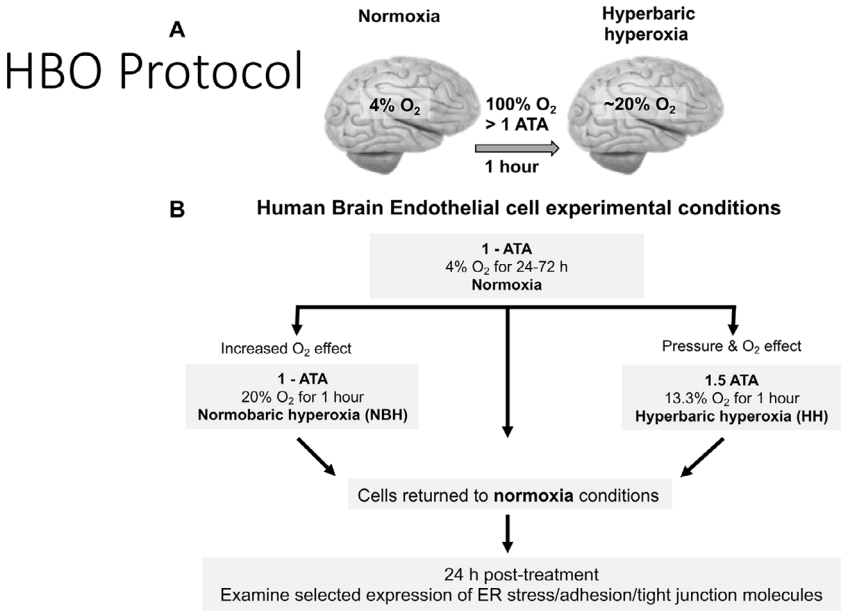

Fig. 4. Protocol design for testing endothelial cell protein expression pre and post oxygen treatment. .

is regulated by adhesion/tight junction molecules and cell stress pathways. Some key adhesion molecules thought to influence this process were selected as targets to measure.

Changes in the cell surface expression of several adhesion molecules involved in inflammatory cell recruitment and attachment to endothelial cells (PECAM, VCAM, E-selectin and ICAM), were quantified pre- and post-oxygen treatments by flow cytometry and immunofluorescence. There was no change in PECAM, VCAM, or E-selectin (Fig. 5). However, ICAM expression appeared sensitive to changes in oxygen levels, and was reduced significantly following treatment with a single exposure of normobaric hyperoxia (increased oxygen) or hyperbaric hyperoxia (increased oxygen and pressure) compared to normoxia oxygen conditions. The reduction was evident from both immunofluorescence and flow cytometry quantification (Fig. 5). The endothelial tight junction protein Zho-1 did not appear to be influenced by oxygen treatment conditions used in this study (data not shown).

3.4. Public involvement group contribution to the design and development of future research

Discussions with the public involvement group over 5-months highlighted the complexities of oxygen therapy for *pw*MS incorporating emotional, physical and practical factors. Several key questions were generated based on their knowledge and conversations with other members of the MS centre which could contribute to the development of an interview guide (see Table 1).

At the final public involvement group meeting, scientific staff joined the group to discuss the provisional findings of the laboratory-based work. In accordance with the groups' preferences a paper combining the scientific and public involvement elements was drafted and all

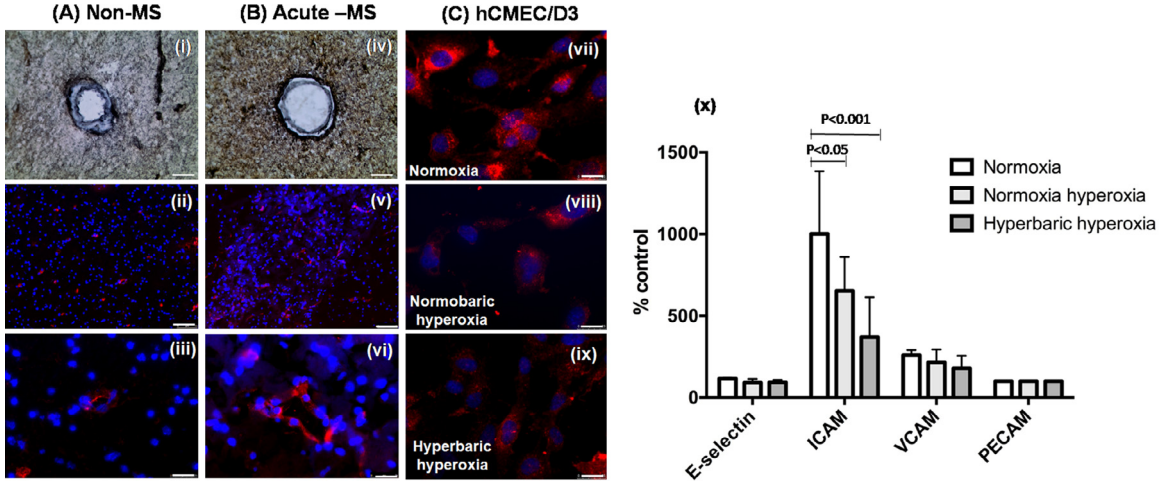

Fig. 5. ICAM expression in MS brain and the effect of oxygen therapy on brain endothelial cell adhesion protein expression. (A) Non-MS brain. (i) Expression of ICAM (brown stain) in cross sectional and longitudinal blood vessels (size bar = 100 μm). (ii & iii) Staining for ICAM (red) in non-MS white matter at x10 and x40 magnification respectively. (B) MS acute brain lesion. (iv) Cross section of blood capillary stained for ICAM. The brain endothelial cells are strongly stained for ICAM, in addition neural cells in the brain parenchyma of MS patients are positive for ICAM (size bar = 100 μm). (v & vi) Staining for ICAM (red) in MS acute lesion white matter at x10 and x40 magnification respectively. Size bars = 25 μm (C) Cell surface ICAM expression under (vii) normoxia conditions diminishes in HCMECs when exposed to oxygen therapy treatment under (viii) normobaric hyperoxia, or (ix) hyperbaric hyperoxia conditions. Size bars = 25 μm. (x) Flow cytometry quantification of reduction in ICAM protein expression compared to other adhesion molecules in cells under normoxic or hyperbaric hyperoxia compared to normobaric normoxia, n = at least 5 separate experiments/condition.

Table 1
Research questions.

Key questions
• **Why** do people with MS choose oxygen therapy? (and who does not choose and why?) • **When** do people with MS choose oxygen therapy? • How do people with MS use oxygen therapy? Prompts: How many sessions, how long, what frequency? • **What** do people with MS feel/experience when receiving oxygen therapy?

In addition, an outline of considerations in relation to aspects of recruitment, method development and data collection in a future research study are summarized in Table 2.

members in the group were given opportunities to read drafts of the paper and make suggestions or amendments.

4. Discussion

It is estimated that over 15,000*pw*MS use the 60 MS national therapy centres each week in and around the UK, Ireland, the Channel islands and Gibraltar (https://www.msntc.org.uk/about-the-centres/). Little is known about the real world experiences of *pw*MS who use oxygen therapy and quantitative methods alone do not always capture aspects of MS accurately or emphasise the most important effects of the disease (Duddy et al., 2014). Patients with worsening symptoms have unmet needs (Nazareth et al., 2018) and may seek help from oxygen therapy (Bogosian et al., 2019). Many studies inadequately report patients' perspectives on quality of life with MS and decisions about their care (Gold et al., 2016).

This study aimed to explore our understanding of how and why pwMS use oxygen therapy. Our research valued and prioritized the public involvement group knowledge and expertise and helped guard against undeveloped and inadequate research (Greenhalgh et al., 2019; Locock et al., 2017) in applied health research teams. These members attending an MS centre for oxygen therapy increased our awareness of the realities and practicalities of oxygen therapy and improved the feasibility and efficacy of the design and development of our present study and future research. Many of these centres directors/trustees are *pw*MS and work in close association with trained healthcare workers. Yet historically, there appears to be a disconnect between the clinical community and *pw*MS who use oxygen therapy on a self-referral basis (Neubauer et al., 2005). The symptoms of MS are highly variable, making the assessment of oxygen therapy difficult in a disease that fluctuates between flares and remission. Reported benefits may be dismissed as a "placebo effect" or, attributed to *pw*MS entering a phase of remission during therapy. A key point that became evident from this study was that the vast majority of clinical trials studies on HBO for *pw*MS have used a treatment regimen of 1.75 – 2.75 ATA, 5 days per week for four weeks. Interaction with our public involvement group has driven us to investigate 1.5 ATA as the most common treatment pressure used. In addition, our lab based cellular studies focus on a single exposure, whereas, *pw*MS undertake oxygen therapy in a variety of patterns - once/twice per week. In future trials it may be more relevant to replicate this practice than reporting on 'one-off' treatments. Treating cells for up to three weeks to assess sustained or transient effects on selected neural cell types, would mirror the *pw*MS protocol more closely.

Despite the controversy, both *pw*MS and the clinical and scientific community agree that more information is necessary on the mode of action of oxygen treatment on the CNS. In vitro experimentation can be informative in this respect. However documented cell-based experimentation often use oxygen conditions that do not reflect what *pw*MS are exposed too physiologically. For example, in many BBB-endothelial cell and ischaemia studies, the cells *in vitro* are exposed to 21% oxygen which reflects normobaric hyperoxia, rather than normobaric normoxia (Patabendige et al., 2018; Shi et al., 2018). At the gene expression level, human umbilical vein endothelial cells (HUVECs) are exquisitely sensitive not only to oxygen but also changes in pressure, with microvascular endothelial cells altering gene expression of key transcription factors and oxidative stress, growth and maturation genes upon one or two exposures of oxygen therapy (Godman et al., 2010) and inflammation (Kendall et al., 2012). It is therefore important for both *pw*MS and scientist to know what effect exposing the CNS to hyperbaric hyperoxia on a regular basis has, which is highlighted in the perceptions by *pw*MS illustrated in Fig. 2. It was of interest that when discussing the use of oxygen therapy, members of the public involvement group highlighted both positive and negative concerns of using oxygen therapy and even the use of the term 'hyperbaric'. Despite safety concerns oxygen therapy is used clinically for series conditions such as brain trauma (Hadanny and Efrati, 2016) and in a retrospective study of ~2300 subjects, oxygen therapy under hyperbaric conditions was considered one of the safest 'medical' treatments (Hadanny et al., 2016), notwithstanding observing similar relatively minor complications such as ear pain, anxiety and dizziness. These symptoms were experienced by the public involvement group in this study and influenced some members decisions to use oxygen therapy.

Since as early as the 1990's there has been an interest in the expression of adhesion molecules on the surface of brain endothelial cells in MS patients (Raine et al., 1990). This is due in part to adhesion molecules such as ICAM acting as a homing molecule for inflammatory T-cells to cross the BBB (Tsukada et al., 1993a). ICAM on cells and in a soluble form (sICAM) in the CSF is proposed as an immunologic biomarker of the clinical activity of MS (Tsukada et al., 1993b). Adhesion molecules in general are known to be important in the treatment of MS – with the biological natalizamab being used previously to inhibit α4 integrins on immune cells therefore preventing their movement across the BBB (Steinman, 2005).

Other adhesion molecules are being investigated to prevent inflammatory T-cells crossing the BBB. In this respect ICAM is a possible therapeutic target. However, increased expression of ICAM is often regarded as a signature of the inflammatory process (Marrosu et al.,

Table 2
Research development and design generated from PPI meetings.

Recruitment process and sample	Design, development and data collection	Method development
• Potential recruitment of 140 members • Advertise study through centre rather than via researchers • Dropout rate up to 50% after several months (consider effect on participants and results) • Majority of members at later stages of MS (consider implications for recruitment and participant sample) • Oxygen therapy accessed privately not prescribed. Members inform doctor of intention to attend centre (consider how to manage informing clinical staff)	• Flexible use of chambers and pressure (consider managing data collection to suit participants preferences and availability) • Treatment of other medical conditions and use of other therapies at centre • Members have other commitments (work), distance of travel and symptoms of fatigue (consider burden of participation) • Type of MS not always known (consider how to gather this information and from whom, with consent from participants)	• Qualitative semi-structured interviews developed around mind map and research questions • Include Spouses/relatives perceptions of the effects of oxygen therapy

2000). Therefore, is it possible that HBO might alleviate inflammatory symptoms of MS rather than prevent transmigration of immune cells into the brain. Our current findings reveal that ICAM expression is lower on hCMEC/D3 cells post hyperbaric oxygen exposure. This supports previous data where hyperbaric oxygen was shown to inhibit ICAM-1 expression via promoting endothelial cell nitric oxide synthase (eNOS) production (Buras et al., 2000). However, our own previous work showed that neutrophil adhesion to HUVECs was inhibited by HBO in vitro, via induction of induced nitric oxide synthase (iNOS) and subsequent S-nitrosation of cellular proteins (Kendall et al., 2013). Similarly, a recent study also showed that neutrophil adhesion to endothelial cells was inhibited in ischaemic reperfusion injuries by inducing nitric oxide and inhibiting CD18 + ve neutrophil - $ICAM^{+ve}$ endothelial adhesion (Francis et al., 2017). Neutrophils have been proposed to be involved in the inflammation of MS (Woodberry et al., 2018) and neutrophil-myeloid cell and neutrophil- lymphocyte ratios in the peripheral blood of MS patients are indicative of disease severity including fatigue, depression and anxiety (D'Amico et al., 2019; Hemond et al., 2019). These recent findings together with the ability of oxygen therapy to influence neutrophil and endothelial interactions infers hyperbaric oxygen may influence peripheral innate immune systems which is known to be sensitive to oxygen changes (Walmsley et al., 2008).

5. Conclusions and future directions

This collaboration with a public involvement group, clinicians and scientists generated ideas about how hyperbaric oxygen therapy could lessen the symptoms of MS. While many *pw*MS use oxygen therapy regularly, a strong functional link with disease improvement is still missing. One significant deficit in this regard is the limited number of laboratory-based studies employing the hyperbaric oxygen conditions commonly experienced by *pw*MS together with the culturing of cells under normoxia (4% O_2) as opposed to hyperoxia (21% O_2). This initial study has enabled our exploration into the manipulation of brain cells by oxygen therapy under conditions experienced by individuals rather than those that are technically convenient in the laboratory environment. Our current knowledge about the mode of action of hyperbaric oxygen therapy is restricted and still in its infancy. By engaging in multidisciplinary discussions with many members of an MS centre and the biomedical community, the beneficial role of oxygen therapy and some of the concerns may become evident. We would recommend public involvement at a very early stage in research development. We have found the use of the combined public involvement group approach and experimental methods, based on their advice, strengthened the relevance and research process. A limitation of this study was that we only involved one centre. Furthermore, additional value would be gained if accurate demographic and clinical records were available at each MS centre of a) the numbers of clinically diagnosed MS subjects using oxygen therapy, b) stage of disease at first use; c) frequency and duration of use; c) documented better/worse or no change in symptoms. We would encourage future research to involve *pw*MS in multiple centres to expand further research encompassing the independent and complementary information which is important in conducting research into the treatment of MS.

CRediT authorship contribution statement

Lucy Moore: Conceptualization, Data curation, Validation, Writing - original draft, Writing - review & editing, Funding acquisition, Formal analysis, Investigation. **Paul Eggleton:** Conceptualization, Data curation, Validation, Writing - original draft, Writing - review & editing, Funding acquisition, Project administration, Resources, Supervision, Formal analysis. **Gary Smerdon:** Conceptualization, Methodology, Validation, Writing - original draft, Resources. **Jia Newcombe:** Resources, Writing - original draft, Visualization, Formal analysis. **Janet E. Holley:** Conceptualization, Writing - original draft, Visualization. **Nicholas J. Gutowski:** Conceptualization, Data curation, Validation, Writing - original draft, Writing - review & editing, Funding acquisition, Project administration, Resources, Supervision. **Miranda Smallwood:** Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing - original draft.

Declaration of Competing Interest

None.

Acknowledgments

The authors would like to acknowledge the contributions of the South-West Multiple Sclerosis Therapy Centre, Exeter https://www.southwestmscentre.co.uk, especially the staff and individuals with MS who agreed to participate in the public involvement group portion of this work. This study was supported by a research grant 1708484 from the Royal Devon and Exeter Foundation Trust (PE, LEM & NJG). We would like to thank the technical staff at the DDRC Healthcare Plymouth, UK, for preparing the various gas mixtures required for this study. Tissue samples were supplied by NeuroResource, UCL Institute of Neurology, and the Multiple Sclerosis Society Tissue Bank, funded by the Multiple Sclerosis Society of Great Britain and Northern Ireland, registered charity 207495.

References

Bennett, M., Heard, R., 2010. Hyperbaric oxygen therapy for multiple sclerosis. CNS Neurosci. Ther. 16 (2), 115–124.
Bogosian, A., Morgan, M., Moss-Morris, R., 2019. Multiple challenges for people after transitioning to secondary progressive multiple sclerosis: a qualitative study. BMJ Open 9 (3), e026421.
Boschetty, V., Cernoch, J., 1970. [Use of hyperbaric oxygen in various neurologic diseases. (Preliminary report)]. Bratisl. Lek. Listy 53 (3), 298–302.
Brett, J., Staniszewska, S., Mockford, C., Herron-Marx, S., Hughes, J., Tysall, C., Suleman, R., 2014a. Mapping the impact of patient and public involvement on health and social care research: a systematic review. Health Expect 17 (5), 637–650.
Brett, J., Staniszewska, S., Mockford, C., Herron-Marx, S., Hughes, J., Tysall, C., Suleman, R., 2014b. A systematic review of the impact of patient and public involvement on service users, researchers and communities. Patient 7 (4), 387–395.
Buras, J.A., Stahl, G.L., Svoboda, K.K., Reenstra, W.R., 2000. Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. Am. J. Physiol. Cell Physiol. 278 (2), C292–C302.
Burki, T., 2019. 2019 Nobel Prize awarded for work on oxygen regulation. Lancet 394 (10207), 1399–1400.
Carreau, A., El Hafny-Rahbi, B, Matejuk, A., Grillon, C., Kieda, C., 2011. Why is the partial oxygen pressure of human tissues a crucial parameter? Small molecules and hypoxia. J. Cell Mol. Med. 15 (6), 1239–1253.
D'Amico, E., Zanghi, A., Romano, A., Sciandra, M., Palumbo, G.A.M., Patti, F., 2019. The Neutrophil-to-Lymphocyte Ratio is Related to Disease Activity in Relapsing Remitting Multiple Sclerosis. Cells 8 (10).
Daugherty, W.P., Levasseur, J.E., Sun, D., Rockswold, G.L., Bullock, M.R., 2004. Effects of hyperbaric oxygen therapy on cerebral oxygenation and mitochondrial function following moderate lateral fluid-percussion injury in rats. J. Neurosurg. 101 (3), 499–504.
Duddy, M., Lee, M., Pearson, O., Nikfekr, E., Chaudhuri, A., Percival, F., Roberts, M., Whitlock, C., 2014. The UK patient experience of relapse in Multiple Sclerosis treated with first disease modifying therapies. Mult. Scler. Relat. Disord. 3 (4), 450–456.
Eggleton, P., 2016. Multiple Sclerosis Survivors Swear By Hyperbaric Oxygen – but does It work?, The Conversation, 1 September, 2016 ed. The Conversation Trust (UK) Limited. https://theconversation.com/multiple-sclerosis-survivors-swear-by-hyperbaric-oxygen-but-does-it-work-64405.
Eggleton, P., Smerdon, G.R., Holley, J.E., Gutowski, N.J., 2017. Manipulation of oxygen and endoplasmic reticulum stress factors as possible interventions for treatment of multiple sclerosis: evidence for and against. Adv. Exp. Med. Biol. 958, 11–27.
Evans, S.M., Judy, K.D., Dunphy, I., Jenkins, W.T., Hwang, W.T., Nelson, P.T., Lustig, R.A., Jenkins, K., Magarelli, D.P., Hahn, S.M., Collins, R.A., Grady, M.S., Koch, C.J., 2004. Hypoxia is important in the biology and aggression of human glial brain tumors. Clin. Cancer Res. 10 (24), 8177–8184.
Faulkner, A., 2013. Exploring the impact of publich involvement on the quality of research: examples. NIHR publication, Hampshire, UK, pp. 1–30. https://www.invo.org.uk/wp-content/uploads/2014/11/INVOLVEExploringImpactQualityResearchExamples_updated-July-2014.pdf.
Fischer, B.H., Marks, M., Reich, T., 1983. Hyperbaric-oxygen treatment of multiple

sclerosis. A randomized, placebo-controlled, double-blind study. N. Engl. J. Med. 308 (4), 181–186.
Francis, A., Kleban, S.R., Stephenson, L.L., Murphy, P.S., Letourneau, P.R., Fang, X.H., Wang, W.Z., Baynosa, R.C., 2017. Hyperbaric Oxygen Inhibits Reperfusion-Induced Neutrophil Polarization and Adhesion Via Plasmin-Mediated VEGF Release. Plast. Reconstr. Surg. Glob. Open 5 (9), e1497.
Godman, C.A., Chheda, K.P., Hightower, L.E., Perdrizet, G., Shin, D.G., Giardina, C., 2010. Hyperbaric oxygen induces a cytoprotective and angiogenic response in human microvascular endothelial cells. Cell Stress Chaperones 15 (4), 431–442.
Gold, R., Toumi, M., Meesen, B., Fogarty, E., 2016. The payer's perspective: what is the burden of MS and how should the patient's perspective be integrated in health technology assessment conducted for taking decisions on access to care and treatment? Mult. Scler. 22 (2 Suppl), 60–70.
Gordon, L., Dickinson, A., Offredy, M., Smiddy, J., 2017. A research note on the benefit of patient and public involvement in research: the experience of prostate cancer patients regarding information in radiotherapy. Radiography (Lond) 23 (2), 167–170.
Green, G., 2016. Power to the people: to what extent has public involvement in applied health research achieved this? Res. Involv. Engagem. 2, 28.
Greenhalgh, T., Hinton, L., Finlay, T., Macfarlane, A., Fahy, N., Clyde, B., Chant, A., 2019. Frameworks for supporting patient and public involvement in research: systematic review and co-design pilot. Health Expect 22 (4), 785–801.
Hadanny, A., Efrati, S., 2016. The efficacy and safety of hyperbaric oxygen therapy in traumatic brain injury. Expert Rev. Neurother. 16 (4), 359–360.
Hadanny, A., Meir, O., Bechor, Y., Fishlev, G., Bergan, J., Efrati, S., 2016. The safety of hyperbaric oxygen treatment–retrospective analysis in 2,334 patients. Undersea Hyperb. Med. 43 (2), 113–122.
Haile, Y., Deng, X., Ortiz-Sandoval, C., Tahbaz, N., Janowicz, A., Lu, J.Q., Kerr, B.J., Gutowski, N.J., Holley, J.E., Eggleton, P., Giuliani, F., Simmen, T., 2017. Rab32 connects ER stress to mitochondrial defects in multiple sclerosis. J. Neuroinflamm. 14 (1), 19.
Hemond, C.C., Glanz, B.I., Bakshi, R., Chitnis, T., Healy, B.C., 2019. The neutrophil-to-lymphocyte and monocyte-to-lymphocyte ratios are independently associated with neurological disability and brain atrophy in multiple sclerosis. BMC Neurol. 19 (1), 23.
Holley, J.E., Bremer, E., Kendall, A.C., de Bruyn, M., Helfrich, W., Tarr, J.M., Newcombe, J., Gutowski, N.J., Eggleton, P., 2014. CD20+inflammatory T-cells are present in blood and brain of multiple sclerosis patients and can be selectively targeted for apoptotic elimination. Mult. Scler. Relat. Disord. 3 (5), 650–658.
James, P.B., 2017a. Oxygen and the Brain: The Journey of Our Lifetime. Best Publishing Company, Florida, USA.
James, P.B., 2017b. Oxygen treatment for multiple sclerosis. In: Jain, K.K. (Ed.), Textbook of Hyperbaric Medicine. Springer, Switzerland, pp. 341–350.
Jung, J., Eggleton, P., Robinson, A., Wang, J., Gutowski, N., Holley, J., Newcombe, J., Dudek, E., Paul, A.M., Zochodne, D., Kraus, A., Power, C., Agellon, L.B., Michalak, M., 2018. Calnexin is necessary for T cell transmigration into the central nervous system. JCI Insight 3 (5), e98410 https://doi.org/98410.91172/jci.insight.98410.
Kendall, A.C., Whatmore, J.L., Harries, L.W., Winyard, P.G., Smerdon, G.R., Eggleton, P., 2012. Changes in inflammatory gene expression induced by hyperbaric oxygen treatment in human endothelial cells under chronic wound conditions. Exp. Cell Res. 318 (3), 207–216.
Kendall, A.C., Whatmore, J.L., Winyard, P.G., Smerdon, G.R., Eggleton, P., 2013. Hyperbaric oxygen treatment reduces neutrophil-endothelial adhesion in chronic wound conditions through S-nitrosation. Wound Repair Regen 21 (6), 860–868.
Kirby, J.P., Snyder, J., Schuerer, D.J.E., Peters, J.S., Bochicchio, G.V., 2019. Essentials of Hyperbaric Oxygen Therapy: 2019 Review. Mo Med. 116 (3), 176–179.
Kochhhar, R.R., Sangwan, A., 2014. Multiple Sclerosis and hyperbaric oxygen therapy: looking back to move ahead (1969-2013). Ind. J. Aerospace Med. 58 (1), 27–38.
Locock, L., Boylan, A.M., Snow, R., Staniszewska, S., 2017. The power of symbolic capital in patient and public involvement in health research. Health Expect 20 (5), 836–844.
Long, Y., Tan, J., Nie, Y., Lu, Y., Mei, X., Tu, C., 2017. Hyperbaric oxygen therapy is safe and effective for the treatment of sleep disorders in children with cerebral palsy. Neurol. Res. 39 (3), 239–247.
Mann, C., Chilcott, S., Plumb, K., Brooks, E., Man, M.S., 2018. Reporting and appraising the context, process and impact of PPI on contributors, researchers and the trial during a randomised controlled trial - the 3D study. Res. Involv. Engagem. 4, 15.
Marrosu, M.G., Schirru, L., Fadda, E., Mancosu, C., Lai, M., Cocco, E., Cucca, F., 2000. ICAM-1 gene is not associated with multiple sclerosis in sardinian patients. J. Neurol. 247 (9), 677–680.
Namazi, H., 2008. Decreasing the expression of LFA-1 and ICAM-1 as the major mechanism for the protective effect of hyperbaric oxygen on ischemia-reperfusion injury. Microsurgery 28 (4), 300.
Nazareth, T.A., Rava, A.R., Polyakov, J.L., Banfe, E.N., Waltrip Ii, R.W., Zerkowski, K.B., Herbert, L.B., 2018. Relapse prevalence, symptoms, and health care engagement: patient insights from the Multiple Sclerosis in America 2017 survey. Mult. Scler. Relat. Disord. 26, 219–234.
Neubauer, R.A., Neubauer, V., Gottleb, S.F., 2005. The controversy over hyperbaric oxygenation therapy for Multiple Sclerosis. J. Am. Phys. Surg. 10 (4), 1125 -1115.
Ocloo, J., Garfield, S., Dawson, S., Dean Franklin, B., 2017. Exploring the theory, barriers and enablers for patient and public involvement across health, social care and patient safety: a protocol for a systematic review of reviews. BMJ Open 7 (10), e018426.
Oscarsson, N., Muller, B., Rosen, A., Lodding, P., Molne, J., Giglio, D., Hjelle, K.M., Vaagbo, G., Hyldegaard, O., Vangedal, M., Salling, L., Kjellberg, A., Lind, F., Ettala, O., Arola, O., Seeman-Lodding, H., 2019. Radiation-induced cystitis treated with hyperbaric oxygen therapy (RICH-ART): a randomised, controlled, phase 2-3 trial. Lancet Oncol. 20 (11), 1602–1614.
Pandya-Wood, R., Barron, D.S., Elliott, J., 2017. A framework for public involvement at the design stage of NHS health and social care research: time to develop ethically conscious standards. Res. Involv. Engagem. 3, 6.
Patabendige, A., Michael, B.D., Craig, A.G., Solomon, T., 2018. Brain microvascular endothelial-astrocyte cell responses following Japanese encephalitis virus infection in an in vitro human blood-brain barrier model. Mol. Cell Neurosci. 89, 60–70.
Puts, M.T.E., Sattar, S., Ghodraty-Jabloo, V., Hsu, T., Fitch, M., Szumacher, E., Ayala, A.P., Alibhai, S.M.H., 2017. Patient engagement in research with older adults with cancer. J. Geriatr. Oncol. 8 (6), 391–396.
Raine, C.S., Lee, S.C., Scheinberg, L.C., Duijvestin, A.M., Cross, A.H., 1990. Adhesion molecules on endothelial cells in the central nervous system: an emerging area in the neuroimmunology of multiple sclerosis. Clin. Immunol. Immunopathol. 57 (2), 173–187.
Rockswold, S.B., Rockswold, G.L., Zaun, D.A., Zhang, X., Cerra, C.E., Bergman, T.A., Liu, J., 2010. A prospective, randomized clinical trial to compare the effect of hyperbaric to normobaric hyperoxia on cerebral metabolism, intracranial pressure, and oxygen toxicity in severe traumatic brain injury. J. Neurosurg. 112 (5), 1080–1094.
Ross, F., Donovan, S., Brearley, S., Victor, C., Cottee, M., Crowther, P., Clark, E., 2005. Involving older people in research: methodological issues. Health Soc. Care Commun. 13 (3), 268–275.
Shi, Y., Lin, H., Cao, J., Cui, C., 2018. Botulinum toxin type A induces protective autophagy in human dermal microvascular endothelial cells exposed to an in vitro model of ischemia/reperfusion injury. Exp. Ther. Med. 16 (6), 4379–4386.
Song, K., Zhang, M., Liu, Y., Wang, Y., Ma, X., 2016. [The effect of hyperbaric oxygen preconditioning on the expression of ICAM-1, VCAM-1, NF-kappaB and flap survival rate during ischemia-reperfusion injury in rat abdominal skin flap]. Zhonghua Zheng Xing Wai Ke Za Zhi 32 (3), 203–207.
Staniszewska, S., Haywood, K.L., Brett, J., Tutton, L., 2012. Patient and public involvement in patient-reported outcome measures: evolution not revolution. Patient 5 (2), 79–87.
Staniszewska, S., Jones, N., Newburn, M., Marshall, S., 2007. User involvement in the development of a research bid: barriers, enablers and impacts. Health Expect 10 (2), 173–183.
Steinman, L., 2005. Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab. Nat. Rev. Drug Discov. 4 (6), 510–518.
Tanaka, T., Nitta, Y., Morimoto, K., Nishikawa, N., Nishihara, C., Tamada, S., Kawashima, H., Nakatani, T., 2011. Hyperbaric oxygen therapy for painful bladder syndrome/interstitial cystitis resistant to conventional treatments: long-term results of a case series in Japan. BMC Urol. 11, 11.
The Guideline Development Group, 2014. Multiple sclerosis in adults: management. https://www.nice.org.uk/guidance/cg186.
Tsukada, N., Matsuda, M., Miyagi, K., Yanagisawa, N., 1993a. Adhesion of cerebral endothelial cells to lymphocytes from patients with multiple sclerosis. Autoimmunity 14 (4), 329–333.
Tsukada, N., Matsuda, M., Miyagi, K., Yanagisawa, N., 1993b. Increased levels of intercellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor receptor in the cerebrospinal fluid of patients with multiple sclerosis. Neurology 43 (12), 2679–2682.
Walker, J.M., Mulatya, C., Hebert, D., Wilson, S.H., Lindblad, A.S., Weaver, L.K., 2018. Sleep assessment in a randomized trial of hyperbaric oxygen in U.S. service members with post concussive mild traumatic brain injury compared to normal controls. Sleep Med. 51, 66–79.
Walmsley, S.R., McGovern, N.N., Whyte, M.K., Chilvers, E.R., 2008. The HIF/VHL pathway: from oxygen sensing to innate immunity. Am. J. Respir. Cell Mol. Biol. 38 (3), 251–255.
Whiting, M., Sines, D., 2012. Mind maps: establishing 'trustworthiness' in qualitative research. Nurse Res 20 (1), 21–27.
Woodberry, T., Bouffler, S.E., Wilson, A.S., Buckland, R.L., Brustle, A., 2018. The Emerging Role of Neutrophil Granulocytes in Multiple Sclerosis. J. Clin. Med. 7 (12).